Pharmacology Biochemistry & Behavior, Vol. 19, pp. 27–32, 1983.

Interaction Between Noradrenergic and Serotonergic Brain Systems as Evidenced by Behavioral and Biochemical Effects of Microinjections of Adrenergic Agonists and Antagonists into the Median Raphe Nucleus

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Received 8 September 1982

PŁAŹNIK, A., W. DANYSZ, W. KOSTOWSKI, A. BIDZIŃSKI AND M. HAUPTMANN. *Interaction between noradrenergic and serotonergic brain systems as evidenced by behavioral and biochemical effects of microinjections of adrenergic agonists and antagonists into the median raphe nucleus.* PHARMACOL BIOCHEM BEHAV **19**(1) 27–32 1983.—The effects of microinjections of adrenergic receptors agonists and antagonists into the median raphe nucleus (MR) on behavior and serotonin (5HT) metabolism was examined in rats. Administration of adrenergic alpha$_1$ and alpha$_2$ receptor agonists (noradrenaline, phenylephrine, clonidine) produced behavioral excitation in the open field test and a tendency to decrease the forebrain 5-hydroxyindolo-acetic acid (5HIAA) concentration. Opposite effects were seen after microinjection of adrenergic alpha receptor antagonists (phenoxybenzamine, phentolamine but not yohimbine). A significant negative correlation was found between the effects on locomotor activity and 5HIAA levels in these rats. No effect was present after injection of beta receptor agonist salbutamol or antagonist propranolol. It is suggested that noradrenaline released from noradrenergic terminals in the MR tonically inhibits the activity of 5HT neurons thus producing symptoms of 5HT deficiency and that this action of noradrenaline is probably limited to the effects on alpha$_1$ but not alpha$_2$ nor beta adrenoceptors in this brain region.

Adrenergic agents Serotonergic median raphe nucleus Microinjection study

SINCE 20 years brain catecholamines (CA) and 5-hydroxytryptamine (serotonin, 5HT) have been commonly believed to be intimately involved in the regulation of behavioral processes as well as of brain bioelectric activity. Moreover, it appeared that the homeostasis of various brain activities depends upon the functional balance in these neurotransmitter systems as well as other putative neurotransmitters and neuromodulators [8, 10, 21].

This assumption was evolved from the finding that pharmacological and surgical manipulations affecting selectively brain 5HT and CA neurons oppositedly influence various behavioral processes such as locomotion, exploratory behavior, aggression and learning [8, 9, 21, 22, 23]. Increasing of CA transmission usually activates various forms of animals behavior, while 5HT mimetic action suppresses them [21].

The anatomical substrates for such interaction have been found in the brainstem CA and 5HT nuclei as well as in the telencephalic terminal areas for axons arising from these loci. The concomittant CA and 5HT innervation was discovered in the brainstem serotonergic dorsal and median raphe nuclei, noradrenergic (NA) locus coeruleus nucleus, dopaminergic (DA) substantia nigra, as well as in the neostriatum, hippocampus, limbic forebrain, cortex and other telencephalic regions [2, 3, 4, 25, 31].

There is many data indicating the functional antagonism between CA and 5HT systems. For example, lesions to the raphe nuclei were shown to produce an increase in forebrain concentration of the main NA metabolite—3,methoxy, 4,hydroxyphenylglycol (MOPEG), as well as antagonized the cataleptic and depressive action of DA receptor blockers—neuroleptics, thus suggesting an opposite role of the raphe nuclei in CA-dependent behavioral and biochemical effects [17, 18, 20]. Similarly, Hollister *et al.*

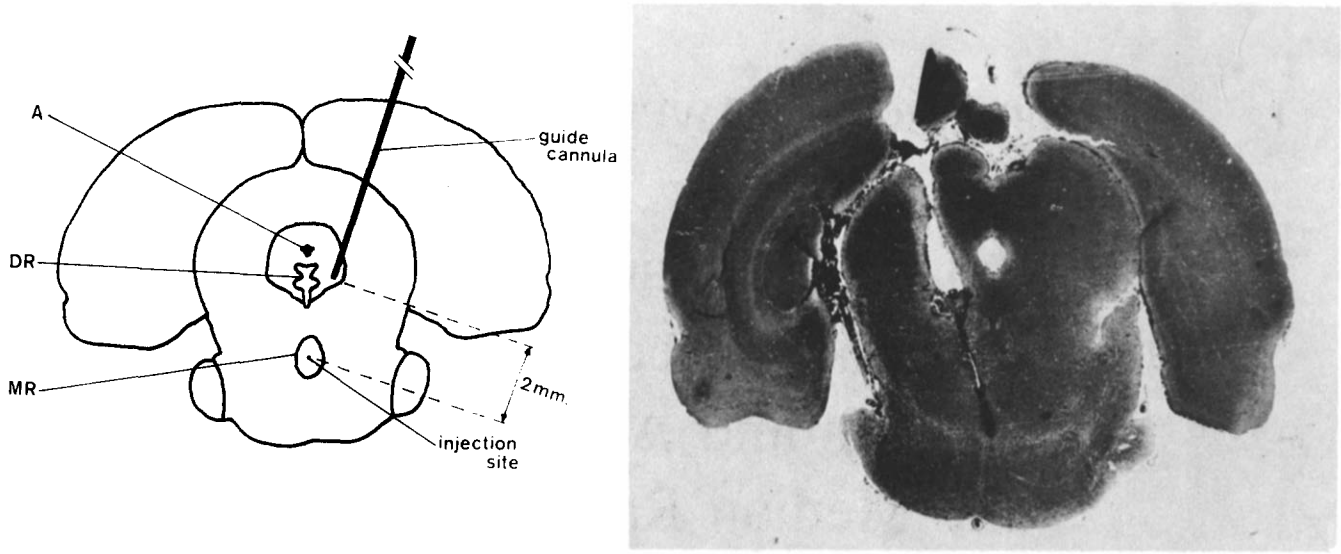

FIG. 1. (A) A schematic drawing showing the place of implantation of the guide cannula and injection of drug solutions. A—aqueduct; DR—dorsal raphe nucleus; MR—median raphe nucleus (B) A photomicrograph showing typical brain damage caused by cannula implantation and microinjections into the MR area.

[13] reported that pretreatment with p-chlorophenylalanine (a 5HT synthesis inhibitor) or 5HT receptor blockers, strongly potentiated the excitement after administration of CA releasing agent—amphetamine. Deficiency of 5HT after median raphe (MR) lesion, produced in the locus coeruleus a significant rise in the concentration of tyrosine hydroxylase, a step limiting enzyme for CA synthesis [26].

In order to get a further insight into the possible NA vs. 5HT interaction, we have analysed some behavioral and biochemical effects of microinjections of NA receptor agonists and antagonists into the median raphe nuclues (MR) of the rat.

METHOD

Male Wistar rats, weighing 180–200 g were used for the experiment. Rats were kept 4–6 to a cage under standard laboratory conditions, with food and water ad lib.

The surgical procedure of cannula implantation was performed as described elsewhere [1]. In short, rats were anesthetized with ether and a guide cannula was then inserted 2 mm above the MR region according to the parameters of the König and Klippel atlas of rat brain [24]: A 0.35 mm, L 0.7 mm, V 3.8 mm, under the angle of 15° to avoid bleeding. The cannula was attached to the skull cap with metal screws and acrylic cement (Fig. 1A). At least one week of recovery was allowed after surgery.

Microinjections of drug solutions were made with 5.0 μl Hamilton microsyringe, in a volume of 0.5 μl during 30 sec. Injection needle protruded 2 mm below the tip of the guide cannula. Each experimental group was injected 2 to 3 times with various doses of the same drug, and at least 4 days of interval between consequent treatment was allowed.

The following drugs were microinjected: noradrenaline bitartrate (Koch Light Lab.), phentolamine chlorhydrate (Ciba-Geigy), phenoxybenzamine chlorhydrate (Smith Kline and French Lab.), clonidine chlorhydrate (Boehringer Ing.), phenylephrine chlorhydrate (Sigma), yohimbine chlorhydrate (Polfa), salbutamol sulphate (Polfa) propranolol chlorhydrate (Polfa). Each solution was prepared immidately before usage, and 0.9% NaCl was applied as a solvent.

Behavioral testing started 30 min after microinjection, in a open field arena (1×1 m), divided into 16 squares—4 central and 12 peripheral. The following parameters were recorded for 5 min: number of crossings of peripheral squares, number of rearings (measure of exploration).

After final injection rats were 30 min latter killed by decapitation and their brains quickly divided by precollicular section into the brainstem and forebrain parts. The brainstems were then fixed in 5% formalin solution and checked for the place of implantation after staining with H and E. The forebrains in turn were analyzed for the concentration of 5,hydroxyindoloacetic acid, (a main 5HT metabolite), according to Haubrich and Denzer [12] and Korf and Sebens [16].

In a separate set of the experiment, the effects of microinjections of drugs with most potent and antagonistic action on behavior (clonidine and phenoxybenzamine) was tested for the changes in 5HT and 5,hydroxyindolacetic acid (5HIAA) levels in the hippocampus and cortex as well as in the "rest" of the telencephalon (mesen-diencephalon), in order to localize more precisely the anatomical substrates for the observed behavioral effects.

Behavioral data were compared with Mann-Whitney nonparametric test, two-tailed, biochemical results with Student *t* test for independent measures, and correlation between behavioral and biochemical changes was done with Spearman correlation test [27].

RESULTS

Out of 120 implanted rats, about 15% was rejected because of incorrect cannula placement, neurological disturbances (ataxia, circling) or cannula clogging. Most rats were

TABLE 1

THE EFFECTS OF MICROINJECTIONS OF ADRENERGIC RECEPTOR AGONISTS INTO THE MEDIAN RAPHE NUCLEUS ON THE BEHAVIOR OF RATS, MEASURED IN THE OPEN FIELD TEST

		peripheral squares	number of rearings
control	n=6	51.8 ± 12.5	16.2 ± 3.5
NA 5 μg	n=6	76.3 ± 8.2*	13.3 ± 3.2
control	n=5	19.8 ± 4.3	7.8 ± 2.5
NA 10 μg	n=6	41.5 ± 5.6‡	11.3 ± 2.1
control	n=6	18.5 ± 1.6	10.2 ± 1.2
Clo. 2 μg	n=6	36.0 ± 1.8*	13.6 ± 1.1
control	n=6	29.8 ± 8.1	12.5 ± 2.5
Clo. 4 μg	n=6	56.7 ± 9.3*	23.2 ± 2.2‡
control	n=6	25.3 ± 7.8	5.2 ± 2.0
Phen. 3 μg	n=6	61.3 ± 8.9*	11.7 ± 4.0
control	n=6	42.0 ± 6.1	10.5 ± 2.3
Phen. 6 μg	n=7	74.0 ± 5.9‡	15.4 ± 1.7†
control	n=5	15.2 ± 7.9	3.0 ± 1.5
S. 6 μg	n=6	17.5 ± 6.6	6.0 ± 1.4

Data are expressed as mean ± SEM.
NA—noradrenaline, Clo.—clonidine, Phen.—phenylephrine, S.—salbutamol.
*=$p<0.05$, †=$p<0.025$, ‡=$p<0.005$, differs from control.

TABLE 2

THE EFFECTS OF MICROINJECTIONS OF ADRENERGIC RECEPTOR ANTAGONISTS INTO THE MEDIAN RAPHE NUCLEUS ON THE BEHAVIOR OF RATS AS MEASURED IN THE OPEN FIELD TEST

		peripheral squares	number of rearings
control	n=7	62.6 ± 9.9	17.3 ± 2.7
Yoh. 2 μg	n=8	85.4 ± 17.8	23.9 ± 3.8
control	n=7	48.7 ± 7.4	9.7 ± 2.1
Yoh. 6 μg	n=8	51.4 ± 15.2	9.9 ± 1.6
control	n=6	27.5 ± 11.0	6.7 ± 2.1
Phenox. 3 μg	n=6	11.3 ± 4.8	2.0 ± 0.7†
control	n=6	120.0 ± 19.7	15.2 ± 2.5
Phenox. 6 μg	n=6	50.2 ± 7.2‡	9.4 ± 1.1
control	n=6	36.2 ± 8.5	10.0 ± 2.1
Phent. 6 μg	n=7	16.0 ± 5.5*	4.3 ± 1.0*
control	n=5	43.4 ± 17.2	13.3 ± 5.4
Phent. 6 μg	n=5	11.2 ± 6.9*	2.8 ± 0.5*
NA 10 μg			
control	n=6	46.8 ± 10.5	12.7 ± 2.2
P. 6 μg	n=7	54.6 ± 13.9	14.3 ± 4.6

Data are expressed as mean ± SEM.
Yoh.—yohimbine, Phenox.—phenoxybenzamine, Phent.—phentolamine, NA—noradrenaline, P.—propranolol. *=$p<0.05$, †=$p<0.025$, ‡=$p<0.005$.

implanted in the MR region, with the anterior-posterior parameter showing the greatest variability (A 0.2–0.4 mm) (Fig. 1).

Microinjection of NA alpha$_1$ and alpha$_2$ receptor agonists: clonidine, noradrenaline and phenylephrine into the MR produced behavioral excitation as shown by increase in number of peripheral squares crossed and number of rearings (Table 1). (Because of variability of behavioral measures between various control groups, resulting from habituation and seasonal changes, we did not pool the results of controls but the data from each control group are shown separately). The most pronounced effect was seen after clonidine and phenylephrine microinjection. These drugs produced also clear tendency to decrease the wholebrain concentration of 5HIAA, but this effect did not reached the significance level (Fig. 3). The treatment with beta$_2$ receptor agonist—salbutamol did not cause any noticeable effect (Table 1).

Opposite behavioral and biochemical effects were observed after injection of NA alpha$_1$ and alpha$_2$ receptor antagonists phenoxybenzamine and phentolamine: behavioral inhibition as evidenced by fall in number of peripheral squares entered as well as in number of rearings (Table 2). Additionally, phentolamine when injected 30 min before NA treatment caused a total antagonism of the excitatory effects of this monoamine on behavior (Table 2).

These drugs seemed to increase also the forebrain 5HIAA concentration, but once again no statistical significance was found (Fig. 3). Phenoxybenzamine produced the most potent changes in behavior. On the other hand alpha$_2$ receptor antagonist yohimbine as well as beta receptor antagonist propranolol were both ineffective neither changing behavior nor 5HIAA levels.

Figures 2 and 3 summarizes the percentage of changes in behavior (peripheral squares) and in 5HIAA levels respectively, after microinjections of NA agonists and antagonists into the MR, as compared with control treated rats. Spearman correlation test showed significant negative correlation between the effects of intraraphe administration of adrenergic agonists and antagonists on behavior (peripheral squares

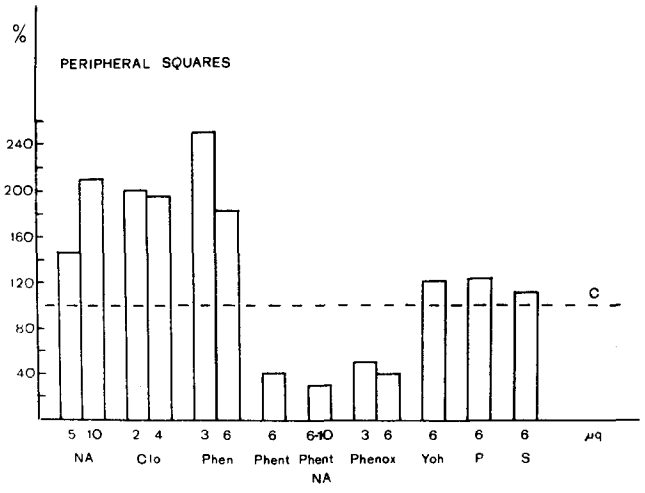

FIG. 2. The summary representation of the effects of microinjections of some adrenergic agonists and antagonists into the MR on behavior (peripheral squares crossed), expressed as a percentage of control rats activity. NA—noradrenaline, Clo—clonidine, Phen—phenylephrine, Phent—phentolamine, Phenox—phenoxybenzamine, Yoh—yohimbine, S—salbutamol, P—propranolol. For further explanations see Tables 1 and 2.

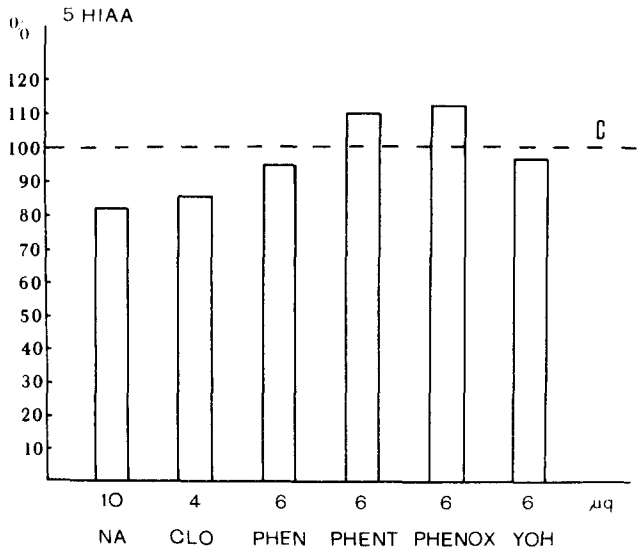

FIG. 3. The summary representation of the effects of some adrenergic agonists and antagonists microinjections into the MR on the wholebrain concentration of 5HIAA, expressed as a percentage of control values. For further explanations see Fig. 2.

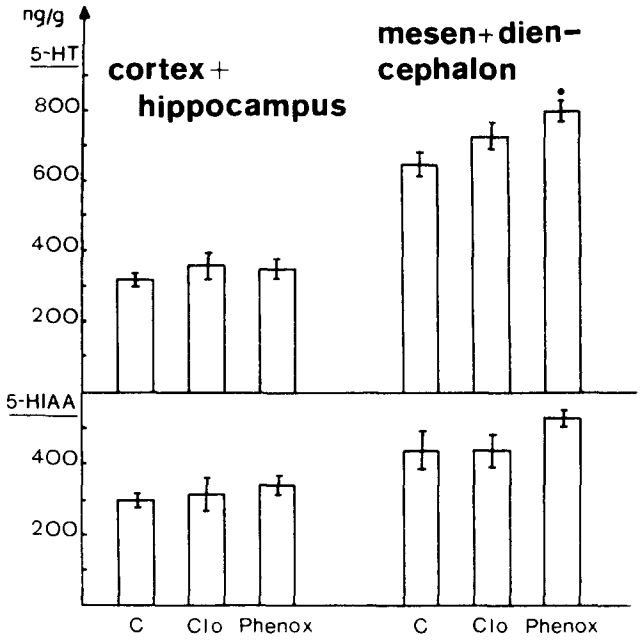

FIG. 4. The effects of microinjections of clonidine and phenoxybenzamine into the MR on the concentration of 5HT and 5HIAA in the cortex + hippocampus and mesen + diencephalon structures of the brain. Data are expressed as mean±SEM. C—control, n=8; Clo—clonidine, 4 μg, n=8; Phenox—phenoxybenzamine, 6 μg, n=8; ●—differs from control rats, $p<0.01$.

crossings) and forebrain 5HIAA levels (n=6, p=−0.94, $p<0.01$).

More detailed examination of changes in forebrain 5HT and 5HIAA levels has revealed that phenoxybenzamine but not clonidine microinjection significantly increased 5HT concentration in the mesen- diencephalon (Fig. 4). No significant variations in 5HIAA levels after such treatment was present (Fig. 4).

DISCUSSION

The present study supports the assumption that microinjections of NA agonists and antagonists into the MR can modulate the activity of 5HT neurons as indicated by changes in animals behavior and forebrain levels of 5HT and 5HIAA. Noradrenaline and NA agonists (e.g., phenylephrine) potentiated locomotor activity and exploration with concomittant tendency to decrease forebrain 5HIAA concentration. On the contrary, NA antagonists (phenoxybenzamine, phentolamine) produced symptoms of behavioral depression and tendency to increase 5HIAA levels in the forebrain. Moreover a significant negative correlation between the effects on behavior and forebrain 5HIAA levels after such treatments was found.

From the other hand it is known that inhibition or stimulation of serotonergic activity of the MR neurons with other methods (electrolytic or chemical lesion, microinjections of 5HT receptor agonists and antagonists) usually results in behavioral and biochemical symptoms closely resembling observed by us effects of microinjections of NA agonists and antagonists respectively [7, 8, 14, 19]. For example, electrolytic lesion to the MR was reported to cause strong behavioral excitation and decrease in forebrain 5HT turnover [29].

Thus the underlying assumption is that microinjection of NA and NA agonists into the MR produces a temporarily hypofunction of 5HT neurons in the MR, followed probably by decreased 5HT turnover in their forebrain terminal areas as evidenced by mentioned above changes in behavioral and biochemical data. Opposite effects should occur after microinjection of NA antagonists into the MR. Indeed, it has been shown by us that phenoxybenzamine, an $alpha_1$ receptor antagonist [6], caused besides its depressant effect on behavior a significant increase in 5HT level in the mesen- diencephalon and tendency to increase the wholebrain 5HIAA concentration. Similarly, a nonselective $alpha_1/alpha_2$ receptor blocker phentolamine [5] induced both: behavioral inhibition and some but insignificant increase in the wholebrain 5HIAA level. Additionally, phentolamine antagonized behavioral excitation after NA microinjection, when preinjected 30 min earlier into the MR.

These results can be interpreted as suggesting the increase in 5HT turnover and greater availability of this monoamine in the forebrain target areas.

Quoted herein results together with the discussed previously data on behavioral and biochemical antagonism between NA and 5HT systems (see introductory paragraphs) may therefore indicate that the MR region can be important for such interaction. We hypothetize that NA released physiologically from the NA terminals in this area tonically inhibits the function of 5HT neurons and as a result modulates the behavioral symptoms related to the function of 5HT neuronal system. Likewisely Kostowski's and Gumulka's [19] and Key's and Kryzywosinski's [15] studies of the effects of microinjections of NA agonists into the dorsal raphe nucleus of the cat on cortical EEG pattern, indicated that dorsal raphe nucleus might receive a NA inhibitory input.

Another notion which can be evolved from our study is that NA released in the MR exerts its inhibitory action upon 5HT neurons probably via stimulation of $alpha_1$ adrenoceptors.

Microinjection of NA as well as pure $alpha_1$ receptor agonist phenylephrine [32], caused similar effects (activation of behavior) contradictory to that produced by $alpha_1$ receptor antagonist phenoxybenzamine. On the other hand yohimbine, an $alpha_2$ receptor antagonist [5,32], was completely ineffective in our hands, thus suggesting the minor role for the $alpha_2$ adrenoceptors in this brain region. The results with clonidine need more careful interpretation. Clonidine preferentially stimulates the $alpha_2$ adrenergic receptors, inhibits NA release from presynaptic stores and thus produces symptoms of behavioral depression [5,32].

In our experimental model this drug caused behavioral and biochemical effects qualitatively similar to that seen after phenylephrine or NA treatment. This contradiction may be explained by the fact that clonidine was found to stimulate also $alpha_1$ receptors under some experimental conditions [34].

The same conclusion can be drawn from the analysis of the effects of phentolamine, a nonselective $alpha_1/alpha_2$ receptor blocker [5]. This drug produced behavioral changes similar to that observed after microinjection of selective $alpha_1$ receptor antagonist—phenoxybenzamine. As in the case of clonidine, the results with phentolamine can be interpreted as further pointing out the predominant role of $alpha_1$ adrenoceptors in the MR nucleus in producing observed by us effects.

The lack of behavioral changes after microinjection of beta adrenoceptor agonists and antagonist: salbutamol and propranolol [28], indicates that this receptor system does not probably occur in the MR or that its function is secondary to or hindered by predominant $alpha_1$ receptor activity. Furthermore it was recently found that β-agonists increase the 5HT turnover after peripheral or intraventricular injection but not after microinjection into the dorsal raphe nucleus [33].

Our results, taking together, point at the specificity of distribution of adrenergic receptors in the MR area. This notion is not probably limited to this brain region only, since different distribution of $alpha_1$ and $alpha_2$ adrenoceptors was found in various structures of the brain [11,30].

In conclusion our data present an evidence for the existence of NA vs. 5HT interaction taking place in the MR nucleus. Noradrenaline release from NA terminals in this area seems to tonically inhibit the activity of 5HT neurons, thus producing symptoms of behavioral excitation and decrease in 5HT turnover rate. This action of NA is probably limited to the effects on $alpha_1$ adrenoceptors in this raphe nucleus.

ACKNOWLEDGEMENTS

The authors wish to thank Koch-Light Lab., Ciba-Geigy, Smith Kline and French Lab., Boehringer Ing., Sigma and Polfa, for their generous supplies of drugs.

REFERENCES

1. Algeri, S., A. Consolazione and A. Płaźnik. Increased metabolism of dopamine and serotonin in forebrain areas induced by etorphine microinjection in periaqueductal gray. *Eur J Pharmacol* **68:** 383–384, 1980.
2. Anderson, C., D. Pasquier, W. Forbes and P. Morgane. Locus coeruleus-to-dorsal raphe input examined by electrophysiological and morphological methods. *Brain Res Bull* **2:** 209–221, 1977.
3. Azmitiá, E. C. The serotonin-producing neurons in the midbrain median and dorsal raphe nuclei. In: *Chemical Pathways in the Brain, Handbook of Psychopharmacology*, vol. 9, edited by L. Iversen, S. Iversen and S. Snyder. New York: Plenum Press, 1978, pp. 223–304.
4. Baraban, J. and G. Aghajanian. Noradrenergic innervation of serotonergic neurons in the dorsal raphe: demonstration by electron microscopic autoradiography. *Brain Res* **204:** 1–11, 1981.
5. Brown, J., J. Doxey and S. Handley. Effects of alpha-adrenoceptor agonists and antagonists and of antidepressant drugs on pre- and postsynaptic alpha-adrenoceptors. *Eur J Pharmacol* **67:** 33–40, 1980.
6. Constantine, J. and W. Lebel. Complete blockade by phenoxybenzamine of $alpha_1$ but not $alpha_2$-vascular receptors in dogs and the effect of propranolol. *Naunyn Schmeidebergs Arch Pharmacol* **314:** 149–156, 1980.
7. Diaz, J., G. Ellison and D. Masuoka. Opposed behavioral syndromes in rats with partial and more complete central serotonergic lesions made with 5,6 dihydroxytryptamine. *Psychopharmacologia* **37:** 67–79, 1974.
8. Ellison, G. Behavior and the balance between norepinephrine and serotonin. *Acta Neurobiol Exp* **35:** 499–515, 1975.
9. Essman, W. B. The role of biogenic amines in memory consolidation. In: *Biology of memory*, edited by G. Adam. Budapest: Akademiai Kiado and New York: Plenum Press, 1971, pp. 213–238.
10. Forchetti, C. and J. Meek. Evidence for a tonic GABA-ergic control of serotonin neurons in the median raphe nucleus. *Brain Res* **206:** 208–212, 1981.
11. Greenberg, D., D. Prichard and S. Snyder. Alpha-noradrenergic receptor binding in mamalian brain: differential binding of agonists and antagonists sites. *Life Sci* **19:** 69–96, 1976.
12. Haubrich, D. and J. Denzer. Simultaneous extraction and fluorometric demeasurement of brain serotonin, catecholamines, 5-hydroxyindoloacetic acid and homovanilic acid. *Anal Biochem* **55:** 306–312, 1973.
13. Hollister, A., G. Breese, C. Moreton, B. Cooper and S Schanberg. An inhibitory role of brain serotonin-containing systems in the locomotory effects of d-amphetamine. *J Pharmacol Exp Ther* **198:** 12–22, 1976.
14. Jacobs, B. and A. Cohen. Differential behavioral effects of lesions of the median and dorsal raphe nuclei in rats: open field and pain elicited aggression. *J Comp Physiol Psychol* **46:** 102–108, 1976.
15. Key, B. and L. Krzywosinski. Electrocortical changes induced by the perfusion of noradrenaline, acetylocholine and their antagonists directly into the dorsal raphe nucleus of the cat. *Br J Pharmacol* **61:** 297–305, 1977.
16. Korf, J. and J. Sebens. Determination of c-conjugated of 5-hydroxytryptamine in humane urine. *Clin Chim Acta* **27:** 149–156, 1970.
17. Kostowski, W., E. Giacalono, S. Garattini and L. Valzelli. Electrical stimulation of midbrain raphe: biochemical, behavioral and bioelectrical effects. *Eur J Pharmacol* **7:** 170–178, 1969.
18. Kostowski, W., W. Gumulka and A. Członkowski. Reduced cataleptogenic effects of some neuroleptics in rats with lesioned midbrain raphe and treated with p-CPA. *Brain Res* **48:** 443–448, 1972.
19. Kostowski, W. and W. Gumulka. Injections of some drugs affecting cholinergic and catecholaminergic neurons into the pontine raphe area. Effect on EEG pattern. *Pol J Pharmacol Pharm* **26:** 351–368, 1974.

20. Kostowski, W., R. Samanin, S. Bareggi, V. Marc, S. Garattini and L. Valzelli. Biochemical aspects of the interaction between midbrain raphe and locus coeruleus in the rat. *Brain Res* **82:** 178–182, 1974.
21. Kostowski, W. Interactions between serotonergic and catecholaminergic systems in the brain. *Pol J Pharmacol Pharam Suppl* **27:** 15–24, 1975.
22. Kostowski, W. Two noradrenergic systems in the brain and their interactions with other monoaminergic neurons. *Pol J Pharmacol Pharam* **31:** 425–436, 1979.
23. Kostowski, W. Noradrenergic interactions among central neurotransmitters. In: *Neurotransmitters, Receptors and Drug Action*, edited by W. Essman. New York: Spectrum, 1980, pp. 47–64.
24. König, J. T. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas of the Forebrain and the Lower Part of the Brainstem*. Baltimore: Williams and Wilkins, 1963.
25. Lindvall, O. and A. Björklund. Organisation of catecholamine neurons in the rat central nervous system. In: *Chemical Pathways in the Brain, Handbook of Psychopharmacology*, vol. 9, edited by L. Iversen, S. Iversen and S. Snyder. New York: Plenum Press, 1978, pp. 139–222.
26. McRae-Degueurce, A., B. Berod, A. Mermet, A. Keller, G. Chouvet, T. Joh and J. Pujol. Alterations in tyrosine hydroxylase activity elicited by raphe nuclei lesions in the rat locus coeruleus: evidence for the involvement of serotonin afferents. *Brain Res* **235:** 285–301, 1982.
27. Meddis, R. *Statistical Handbook for Non-Statisticians*. Bedford College, London University London: Mc Graw Hill, 1975.
28. Nickerson, M. and B. Collier. Drugs inhibiting adrenergic nerves and structures innervated by them. In: *The Pharmacological Basis of Therapeutics*, edited by L. Goodman and A. Gilman. New York: MacMillan, 1975, pp. 533–565.
29. Płaźnik, A., W. Kostowski, A. Bidziński and M. Hauptman. Effects of lesions of the midbrain raphe nuclei on avoidance learning in rats. *Physiol Behav* **24:** 257–262, 1980.
30. Prichard, U., T. Resine, S. Mason, H. Fibiger and H. Yamamura. Modulation of rat brain alpha- and beta- adrenergic receptor populations by lesions of the dorsal noradrenergic bundle. *Brain Res* **187:** 143–154, 1980.
31. Sladek, J. and P. Walker. Serotonin-containing neuronal perikarya in the primate locus coeruleus and subcoeruleus nuclei. *Brain Res* **134:** 359–366, 1977.
32. Starke, K. and T. Endo. Presynaptic alpha-adrenoceptors. *Minireview Gen Pharmacol* **7:** 307–312, 1976.
33. Waldmeier, P. C. Stimulation of central serotonin turnover by β-adrenoceptor agonists. *Naunyn Schmeidebergs Arch Pharmacol* **317:** 115–119, 1981.
34. Żebrowska-Łupina, I., E. Przegaliński, M. Sloniec and Z. Kleinrok. Clonidine-induced locomotor hyperactivity in rats. The role of central postsynaptic alpha-adrenoceptors. *Naunyn Schmeidebergs Arch Pharmacol* **297:** 227–231, 1977.